

C13
final volume of 50 µl containing 0,2mM dNTP, 20mM DTT, 3 µM of each of the 5' and 3' primer, 5 µl of 10x PCR buffer (100 mM Tris-HCl pH 9.0, 500 mM KCl, 1% gelatine, 1,5mM MgCL₂, 1% polyoxyethylene non-ionic detergent TRITON X-100) and 5 U AmpliTaq-DNA-polymerase (Perkin Elmer). PCR was performed with an initial denaturation step of 10 min at 85°C followed by 35 cycles of 20 sec denaturation at 95°C, 30 sec annealing at 60°C and 30 sec extension at 72°C. The amplification products were visualized under a UV lamp after electrophoresis of a 15 µl aliquot of the reaction mixture on a 2% (w/v) agarose gel containing 0,5 µg/ml of ethidium bromide. The primer pair was designed in such a way that the presence of splice products should be indicated by a 190 bp and/or a 125 bp fragment. The identity of the resumable splice product was confirmed by sequencing the fragments after isolation on a preparative agarose gel. The primer paid used for amplification and sequencing - SV40 forward: 5'-GGATCCGGTACTCGAGGAAC-3' (SEQ ID NO:5), SV40 reverse: 5'-GCTTTAGCAGGCTCTTTCG-3' (SEQ ID NO:6).--

In the claims:

- C14
1. [Amended] An attenuated *Salmonella* strain comprising a eukaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment comprised by the vector within an open reading frame wherein the attenuation is suitable for a vaccination of vertebrates.
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4. [Amended] The *Salmonella* strain according to claim 1 wherein the strain is a *S. typhi* strain.
 5. [Amended] The *Salmonella* strain according to claim 4 wherein the strain is *S. typhi* Ty21a.
 - C15
6. [Amended] The *Salmonella* strain according to claim 1 wherein the eukaryotic expression vector is derived from plasmid pCMVB, wherein the plasmid comprises:
 - a) a structural gene of β- galactosidase (β- gal) under the control of a human cytomegalovirus (CMV) immediate early promoter,
 - b) a splice donor,